ANNALS OF SURGERY
Vol. 218, No. 1, 13-21

Immunohistochemical and Biochemical Measurement of Estrogen and Progesterone Receptors in Primary Breast Cancer

Correlation of Histopathology and Prognostic Factors

Michael Stierer,* Harald Rosen,* Renate Weber,† Hanns Hanak,†
Jürgen Spona,§ and Heinz Tüchler‡

From the Department of Surgery, Institute of Histopathology,† and Ludwig Boltzmann Institute for Leukemia Research and Hematology,‡ Hanusch Medical Center, Vienna, and the Endocrinologic Laboratory at the First Department of Gynecology & Obstetrics, and Ludwig Boltzmann Institute for Experimental Endocrinology,§ University of Vienna, Vienna, Austria*

Objective
The authors investigated correlations of estrogen-receptor and progesterone-receptor with conventional risk factors as well as histopathology in patients with primary breast cancer.

Summary Background Data
Immunohistochemically determined hormone receptors have gained importance as prognosticators in primary breast cancer, but their definitive role has not yet been evaluated.

Methods
Tumor samples from 299 patients were examined for estrogen and progesterone receptors by biochemical and immunohistochemical assay. Correlations with established risk factors (tumor size, lymph node status, menopausal status, grading including subfactors) and histopathology were analyzed.

Results
The estrogen receptor, determined by immunohistochemical method revealed positivity in 80.6% of patients; biochemical measurement yielded 76.2% positive results. The progesterone receptor measured by immunohistochemistry yielded 61.3% positivity versus 55.8% detected by biochemical analysis. Invasive lobular, tubular, and ductal invasive carcinoma with prominent stroma content ("scirrhous carcinoma") rather than ductal invasive carcinoma was more frequently estrogen-receptor positive with immunohistochemistry than with biochemical assay. For progesterone receptor, the same pattern of positivity was seen with immunohistochemical assay. With progesterone receptor determined biochemically, "scirrhous" and lobular carcinoma showed positive results in a lower proportion than invasive ductal and tubular carcinoma. Significant correlations were observed between the estrogen-receptor status, the histologic grade of malignancy, nuclear polymorphism, and the rate of mitosis with both methods ($p < 0.001$ respectively). Different correlations were found between tumor size, menopausal status

and estrogen receptor status with both assays respectively. For the progesterone receptor status, immunohistochemistry yielded significant correlations with the histologic grade of malignancy, nuclear polymorphism, rate of mitosis ($p < 0.001$ respectively) as well as growth pattern ($p < 0.01$), while biochemical analysis revealed a correlation with nuclear polymorphism ($p < 0.05$).The correlation analysis of both components of the immunoreactive score revealed a more significant impact of percentage of positive cells than of staining intensity.

Conclusions

Immunohistochemistry detected a closer correlation between prognostic factors and receptor data than biochemical analysis.

Primary breast cancer includes a variety of subgroups with highly variable biologic validity. The following parameters served for prognostic evaluation: locoregional tumor spread, histologic tumor type, malignancy grading, and estrogen (ER) as well as progesterone (PgR) receptors.

While controversial data have been reported with regard to the impact of the biochemically determined estrogen receptor,[1-6] the progesterone receptor is widely acknowledged as a relevant prognostic factor.[7,8] With the recent development of monoclonal antibodies against nuclear estrogen and progestin epitopes[9,10] immunohistochemical measurements have increasingly gained momentum, and several groups have studied their prognostic relevance.[11,12] Clear benefits of this technique are visualization of the receptor protein to disclose tumor heterogeneity, independence of receptor-masking estrogens of endogenous and exogenous origin, no interference of data with receptor-blocking substances, and minimal tumor quantity required for analysis.

The main goal of the ongoing prospective trial was to evaluate the correlation of immunohistochemically determined steroid receptor levels and histopathology. Special emphasis was put on the progesterone receptor determined by immunohistochemistry and the histopathology of the tumor, as scarce pertinent data have been published so far.[11] Another focus of this paper was to study the correlation of both measuring systems with the commonly employed prognostic parameters of breast cancer. In addition, we wanted to assess the role and validity of subfactors applied for immunohistochemical scoring.

MATERIAL AND METHODS

Material for this study was collected from 299 patients with primary breast cancer operated on at the Department of Surgery, Hanusch Medical Center, Vienna. The patients ranged in age between 32 and 92 years (median 66 years). The ratio pre- versus postmenopausal patients was 1:5. The estrogen receptor was determined by immunohistochemistry in all cases, and in 240 cases a biochemical analysis was also performed. Progesterone receptor data by biochemical analysis were available for 242 specimens. Immunohistochemical determination was performed in 168 biopsies due to the only recent availability of the method. In 130 cases, data determined by both assays were available. In 38 patients PgR-DCC determinations had not been performed due to small tumor size or other technical problems.

For biochemical analysis of estrogen and progesterone receptor levels, we used the dextrane-coated charcoal assay (DCC) and Scatchard analysis.[13] A minimum of 0.5 mm^3 of the extirpated tumor was immediately freed from surrounding connective tissue, snapfrozen in liquid nitrogen and stored at $-170°C$. The assay was performed within 1 week past surgery in all cases. The cut off point for positivity/negativity was established at 10 fmol/mg cytosol protein. All biochemical assays were performed by one investigator (JS). The cooperating laboratory complies with the quality requirements of EORTC.

For the immunohistochemical assay (ICA), a slice of tumor tissue was frozen in liquid nitrogen immediately after surgery, cut in 5-μm sections, mounted on glass slides, and fixed in 3.7% phosphate-buffered formaldehyde. All further immunohistochemical staining procedures for estrogen and progesterone receptor analysis were performed according to the instructions for the estrogen receptor (ER-ICA kit, Abbott Diagnostic Division, North Chicago, IL) and the progesterone receptor immunohistochemical assay(PgR-ICA kit, Abbott Diagnostics).

Estrogen and progesterone receptor positive cells provided along with the above test kits served as controls. They were treated with anti-estrogen receptor and anti-progesterone receptor antibody and control antibody, respectively. Specimens yielding positive results with the primary antibody did not show a positive reaction with the control antibody.

Address reprint requests to M. Stierer, Department of Surgery, Hanusch Medical Center, Heinrich Collinstr 30, 1140 Vienna, Austria.
Accepted May 14, 1992.

Classification of staining data with the help of light microscopy was done semiquantitatively by using an immunoreactive score (IRS).[14]

The IRS ranges from 0 to 12 points and is formed by multiplying the values of the subfactors, which are judged independently. The percentage of tumor cells with positive staining (PP) was graded from 0 to 4 points by estimation; 0 points means no positive staining, 1 point is given for less than 10% staining, 2 points represent 11–50% of staining, 3 points 51–80%, and 4 points indicate that more than 80% of tumor nuclei show positive staining. Staining intensity (SI) is scored as 0 = negative, 1 = slight, 2 = moderate, 3 = strong. In case of heterogeneity of SI within the tumor the most predominant staining intensity is scored.

At least 200 cells were counted for each tumor specimen. The results 0 and 1 were considered as receptor negative.

All sections were embedded in paraffin and stained with hematoxylin-eosin for histology. The evaluation was performed in line with the classification of the World Health Organization[15] at 40-fold magnification in the high-power field.

A previous report[16] expressed a certain bias with regard to steroid receptor data due to a prominent stroma content in ductal invasive tumors. This particular feature was classified as "scirrhous carcinoma" and a possible impact in our study was evaluated separately. Other histologic tumor types were excluded because their stroma content may have been related to the tumor type. Staging of tumors was done according to the UICC classification of TNM.[17]

Tumor grading was based on the criteria of Bloom & Richardson.[18] Data obtained by histologic evaluation as well as by immunohistochemical receptor determination were read by two blinded pathologists independently, who were unaware of the results of the biochemical assay procedures. Interobserver reproducibility was 97% for histology and 95.6% for immunohistochemistry.

Lymph node involvement was classified as negative or positive. A positive lymph node status was subdivided into various categories: 1–3 positive nodes, 3–10 positive nodes and 10 or more positive nodes. The tumor diameter was measured on the gross specimen.

Intraductal carcinoma was excluded from this investigation. Patients were defined as postmenopausal when menstruation had been absent for more than a year. None of the patients had previously received hormone receptor blockers.

The following factors were subject to correlation analysis: tumor size, lymph node status, menopausal status, grading including subfactors as well as histologic tumor type.

Table 1. COMPARISON BETWEEN IMMUNOHISTOCHEMICAL AND BIOCHEMICAL ER-STATUS

	ER-DCC	
ER-ICA	Negative	Positive
Negative	32 (13)*	13 (5)
Positive	25 (11)	170 (71)

* Number (%) of tumors.
Total number = 240; Phi-coefficient = 0.53, $p < 0.000001$. TAU-C = 0.44, $p < 0.000001$.

STATISTICAL EVALUATION

For basic data description, the common statistical parameters such as frequency and percentage are given. Correlations were quantified by Kendall's tau-c and tested according to Brown and Benedetti;[20] 2×2 table correlations were quantified by the phi-coefficient. Both coefficients vary within the range of -1 to $+1$. While $+1$ designates full agreement (i.e., positive correlation), a coefficient of -1 reveals full inverse agreement (i.e., negative correlation); 0 expresses no correlation at all. Contingencies in tables of nominal variables were tested by Pearson's chi-square test, in the case of 2×2 tables Yates' correction was applied. All significance tests were performed two-sided at a level of alpha = 5%.

RESULTS

Localization of receptors with ICA was restricted to the nucleus of the tumor cells in all cases of positivity. A total of 76.2% of all samples were estrogen receptor positive versus 55.8% of progesterone receptor positivity as detected by DCC. Immunohistochemistry revealed 80.6% positivity with ER-ICA and 61.3% with PgR-ICA. This corresponds to 84.2% of concordant findings for the estrogen receptor (phi = 0.53, $p < 0.000001$) and 71.3% for the progesterone receptor (phi = 0.41, $p < 0.000001$) (Tables 1–2).

Correlation analysis of the two different assays by use of Kendall's tau-c revealed a highly significant correlation for ER-determination as well as for the PgR-determination (ER: tau-c = 0.44, $p < 0.000001$; PgR: tau-c = 0.35, $p < 0.000001$).

Histologic Tumor Type

The receptor distribution in the respective histologic tumor types is illustrated in Table 3. Mucinous carcinomas were predominantly receptor positive while both

Table 2. COMPARISON BETWEEN IMMUNOHISTOCHEMICAL AND BIOCHEMICAL PGR-STATUS

	PGR-DCC	
PGR-ICA	Negative	Positive
Negative	36 (28)*	15 (11)
Positive	22 (17)	57 (44)

* Number (%) of tumors.
Total number = 130; Phi-coefficient = 0.41, p = <0.000001. TAU-C = 0.35, p = <0.000001.

patients with medullary carcinoma yielded receptor negativity with all methods.

However, the number of these tumor types was too small to yield conclusive results and they were therefore excluded from statistical analysis.

The overall incidence of estrogen positivity was higher with ICA than with DCC; all histologic entities were more commonly receptor positive than the ductal invasive NOS (not otherwise specified) tumors.

On the whole, no significant association existed between estrogen receptor content and tumor type. This was true for both assays (ER-ICA: p = 0.41, ns; ER-DCC: p = 0.63, ns). While the distribution pattern of the various histologic tumor types determined by PgR-ICA was similar to that of the estrogen receptors determined by ER-ICA, PgR-DCC yielded a lower percentage of positivity for the categories 'scirrhous' as well as 'lobular' carcinoma as compared with ductal invasive carcinoma and especially tubular carcinoma, which showed the highest percentage of positivity with all methods. However, the progesterone receptor showed no statistically significant association with a specific histologic tumor type (PgR-ICA: p = 0.17, ns; PgR-DCC: p = 0.22, ns).

Histologic Tumor Grading

There was a highly significant negative correlation between receptor positivity and degree of differentiation. As shown in Table 4, estrogen positivity was more frequent with well-differentiated tumors, as demonstrated with both methods (ER-ICA: tau = −0.26; ER-DCC: tau = −0.24, $p < 0.001$, respectively).

For progesterone receptors, this was true only with PgR-ICA (tau = −0.31, $p < 0.001$) while DCC detected a more even distribution of receptor data (tau = −0.06, p = 0.2, ns). The relationship between each single factor of tumor grading and the receptor content is shown in Tables 5, 6, and 7.

There was no significant correlation between tubular differentiation and estrogen receptor content, irrespective of the employed method (ER-ICA: tau = −0.03, p = 0.25; ER-DCC: tau = −0.09, p = 0.1). The tubular growth pattern was significantly associated with progesterone receptor positivity with ICA (tau = −0.21, p = 0.01), which could not be detected with DCC (tau = −0.0002, p = 1.0).

Estrogen receptor-positive tumors were preferably associated with low-grade nuclear polymorphism (ER-ICA: tau = −0.27; ER-DCC: tau = −0.20, $p < 0.001$, respectively). This was also noted for the progesterone receptor when determined by ICA (tau = −0.32, $p < 0.001$) as well as DCC (tau = −0.13, $p < 0.05$).

There was a significant association of the rate of mitosis and the estrogen receptor content. About 90% of patients with carcinoma exhibiting a low mitotic rate (0–1) were estrogen receptor positive with both methods (ER-ICA: tau = −0.25; ER-DCC: tau = −0.25, $p < 0.001$, respectively).

The progesterone receptor content significantly correlated with the above subfactor only with ICA (tau = −0.30, $p < 0.001$). A rather homogenous distribution

Table 3. RELATIONSHIP BETWEEN RECEPTOR STATUS AND HISTOLOGIC TUMOR TYPE

		ER-ICA		ER-DCC			PGR-ICA		PGR-DCC	
	No. of ER-ICA-DCC	Pos.	Neg.	Pos.	Neg.	No. of PGR-ICA-DCC	Pos.	Neg.	Pos.	Neg.
Invasive ductal NOS	187/150	146 (78)	41 (22)	111 (74)	39 (26)	108/154	60 (56)	48 (44)	90 (58)	64 (42)
"Scirrhous-ca."	63/49	54 (86)	9 (14)	40 (82)	9 (18)	31/48	21 (68)	10 (32)	21 (44)	27 (56)
Lobular ca.	19/15	17 (89)	2 (11)	12 (80)	3 (20)	11/15	9 (82)	2 (18)	8 (53)	7 (47)
Tubular ca.	21/18	18 (86)	3 (14)	15 (83)	3 (17)	13/18	11 (86)	2 (14)	13 (72)	5 (28)
Mucinous ca.	7/6	6	1	5	1	3/5	2	1	3	2
Medullary ca.	2/2	0	2	0	2	2/2	0	2	0	2

Chi-square tests, NS.

Table 4. RELATIONSHIP BETWEEN RECEPTOR STATUS AND HISTOLOGIC TUMOR GRADE

	Grading		
Receptor Status	**1**	**2**	**3**
ER-ICA (N = 299)			
Positive	52 (94%)	141 (86%)	48 (59%)
Negative	3 (6%)	22 (14%)	33 (41%)
ER-DCC (N = 240)			
Positive	39 (89%)	111 (82%)	34 (56%)
Negative	5 (11%)	24 (18%)	28 (44%)
PGR-ICA (N = 168)			
Positive	20 (74%)	65 (71%)	18 (36%)
Negative	7 (26%)	26 (29%)	32 (64%)
PGR-DCC (N = 242)			
Positive	24 (54%)	81 (60%)	30 (48%)
Negative	20 (46%)	54 (40%)	33 (52%)

Table 6. RELATIONSHIP BETWEEN RECEPTOR STATUS AND NUCLEAR POLYMORPHISM

	Nuclear Polymorphism		
Receptor Status	**Low Grade**	**Medium Grade**	**High Grade**
ER-ICA (N = 299)			
Positive	51 (98%)	172 (84%)	18 (43%)
Negative	1 (2%)	33 (16%)	24 (57%)
ER-DCC (N = 240)			
Positive	34 (87%)	138 (80%)	12 (41%)
Negative	5 (13%)	34 (20%)	17 (59%)
PGR-ICA (N = 168)			
Positive	22 (85%)	73 (65%)	8 (27%)
Negative	4 (15%)	39 (35%)	22 (73%)
PGR-DCC (N = 242)			
Positive	24 (61%)	102 (59%)	9 (30%)
Negative	15 (39%)	72 (41%)	20 (70%)

of progesterone receptor data was seen with DCC (tau = −0.10, p = 0.1, ns).

Menopausal Status

As shown in Table 8 the DCC method revealed no significant association between estrogen and progesterone receptor distribution and the menopausal status (ER-DCC: p = 0.41; PgR-DCC: p = 0.67). This is also true for PgR-ICA (p = 1.0, ns) while ER-ICA yielded a significant positive correlation with a trend towards higher positivity in older women (p = 0.01).

Size of Tumor and Lymph Node Status

The percentage of estrogen positive tumors significantly decreased with tumor size (DCC: tau = −0.12, p < 0.05). This was not demonstrated with ER-ICA (tau = −0.05, p = 0.15, ns) The percentage of receptor positive tumors decreased with tumor size with ICA and DCC, although no significant correlation was observed (PgR-ICA: tau = −0.15, p = 0.15; PgR-DCC: tau = −0.06, p = 0.1, ns). There was no statistically significant correlation between lymph node status and both receptors with either method (ER-ICA: tau = −0.02, p = 0.3, ns; ER-DCC: tau = −0.04, p = 0.2 ns; PgR-ICA: tau = −0.01, p = 0.45; PgR-DCC: tau = 0.06, p = 0.2) (Table 9).

Table 5. RELATIONSHIP BETWEEN RECEPTOR STATUS AND TUBULAR DIFFERENTIATION

	Tubular Differentiation		
Receptor Status	**High**	**Intermediate**	**Low**
ER-ICA (N = 299)			
Positive	16 (84%)	95 (82%)	130 (79%)
Negative	3 (16%)	21 (18%)	34 (21%)
ER-DCC (N = 240)			
Positive	11 (92%)	76 (79%)	96 (73%)
Negative	1 (8%)	20 (21%)	36 (27%)
PGR-ICA (N = 168)			
Positive	9 (75%)	50 (71%)	44 (51%)
Negative	3 (25%)	20 (29%)	42 (49%)
PGR-DCC (N = 242)			
Positive	6 (50%)	54 (57%)	75 (56%)
Negative	6 (50%)	41 (43%)	60 (44%)

Table 7. RELATIONSHIP BETWEEN RECEPTOR STATUS AND RATE OF MITOSIS

	Rate of Mitosis		
Receptor Status	**0-1/HPF**	**2/HPF**	**3 or more/HPF**
ER-ICA (N = 299)			
Positive	69 (93%)	128 (86%)	44 (58%)
Negative	5 (7%)	21 (14%)	32 (42%)
ER-DCC (N = 240)			
Positive	54 (90%)	95 (80%)	35 (56%)
Negative	6 (10%)	23 (20%)	27 (44%)
PGR-ICA (N = 168)			
Positive	27 (73%)	61 (71%)	15 (33%)
Negative	10 (27%)	25 (29%)	30 (67%)
PGR-DCC (N = 242)			
Positive	34 (58%)	74 (61%)	27 (43%)
Negative	25 (42%)	47 (39%)	35 (57%)

Table 8. RELATIONSHIP BETWEEN RECEPTOR STATUS AND MENOPAUSAL STATUS

Menopausal Status	No. of ER-ICA-DCC	ER-ICA Pos.	ER-ICA Neg.	ER-DCC Pos.	ER-DCC Neg.	No. of PGR-ICA-DCC	PGR-ICA Pos.	PGR-ICA Neg.	PGR-DCC Pos.	PGR-DCC Neg.
Premenopausal	51/40	34 (60%)	17 (33%)	28 (70%)	12 (30%)	35/40	21 (60%)	14 (40%)	24 (60%)	16 (40%)
Postmenopausal	248/200	207 (83%)	41 (17%)	156 (78%)	44 (22%)	133/202	82 (62%)	51 (38%)	111 (55%)	91 (45%)

RELATIONSHIP BETWEEN SUBFACTORS OF ICA AND PROGNOSTIC FACTORS

Staining Intensity

For the estrogen receptor, there was a highly significant negative correlation of SI and grading, nuclear polymorphism, and rate of mitosis ($p < 0.001$, respectively). On the other hand, there was a significant positive correlation with the menopausal status ($p < 0.01$). For the progesterone receptor, there was a significant negative correlation between SI and grading, nuclear polymorphism, rate of mitosis ($p < 0.001$ respectively), and tubular differentiation ($p < 0.02$).

Percentage of Positive Cells

Estrogen receptor analysis revealed that this subfactor of ICA had a significant negative correlation with grading, nuclear polymorphism, rate of mitosis, and tumor size ($p < 0.001$ respectively), while there was a positive correlation with the menopausal status ($p < 0.002$). Progesterone receptor analysis showed a negative correlation with grading, nuclear polymorphism, rate of mitosis ($p < 0.001$ respectively), and tubular differentiation ($p < 0.002$). Additionally, ductal invasive NOS tumors yielded a significantly higher number of samples with less than 10% positive cells than the other histologic tumor types included in the statistical analysis ($p = 0.01$). There was no correlation of subfactors with any other prognostic factor (data not shown).

DISCUSSION

In our patients, an increased incidence of receptor-positive tumors occurred, especially with regard to estrogen receptors. This may be due to the high percentage of

Table 9. RELATIONSHIP BETWEEN RECEPTOR STATUS AND SIZE OF TUMOR AS WELL AS LYMPH NODE STATUS

	No. of ER-ICA-DCC	ER-ICA Pos.	ER-ICA Neg.	ER-DCC Pos.	ER-DCC Neg.	No. of PGR-ICA-DCC	PGR-ICA Pos.	PGR-ICA Neg.	PGR-DCC Pos.	PGR-DCC Neg.
Tumor size										
T_1	160/122	131 (82%)	29 (18%)	99 (81%)	23 (19%)	88/124	59 (67%)	29 (33%)	73 (59%)	51 (41%)
T_2	116/100	97 (84%)	19 (16%)	76 (76%)	24 (24%)	66/100	39 (59%)	27 (41%)	53 (53%)	47 (47%)
$T_{3,4}$	23/18	13 (56%)	10 (44%)	9 (50%)	9 (50%)	14/18	5 (36%)	9 (64%)	9 (50%)	9 (50%)
Lymph node status										
Negative	183/144	149 (81%)	34 (19%)	113 (78%)	31 (22%)	103/146	63 (61%)	40 (39%)	76 (52%)	70 (48%)
1–3 Pos. nodes	70/59	57 (81%)	13 (19%)	45 (76%)	14 (24%)	40/61	27 (67%)	13 (33%)	42 (69%)	19 (31%)
4–10 Pos. nodes	35/29	26 (74%)	9 (26%)	19 (65%)	10 (35%)	22/27	11 (50%)	11 (50%)	13 (48%)	14 (52%)
More than 10 pos. nodes	11/8	9 (82%)	2 (18%)	7 (87%)	1 (13%)	3/8	2 (67%)	1 (33%)	7 (87%)	1 (13%)

postmenopausal women in this series. The choice of the cut-off point for the IRS estimation with the ICA method will have to be further validated by therapeutic studies, in particular by follow-up trials with long-term observation.

The overall agreement of ER-ICA with ER-DCC in our series essentially corresponds to the recently reported percentage of 84%.[21] For the progesterone receptor, two reports have stated an incidence of positivity of 38 and 48%, respectively.[22,23] In a series investigated by the Finsen Institute, 70% of cases were PgR positive.[24]

The concordant findings we obtained for different methods to determine the progesterone receptor content are in agreement with the literature.[12]

Although correlation analysis of DCC and ICA methods yielded a strong correlation for both receptors in our series, we were not able to show a perfect correlation between the two assays (ER: tau-c = 0.44, $p < 0.000001$; PgR: tau-c = 0,35, $p < 0.000001$). Therefore we believe that due to the different nature of both assays it is not possible to substitute one test by the other at the present time. However one might be able to evaluate a definitive role of the ICA method after prospective trials employing both assays. On the other hand, this will be possible only if a direct, perfect correlation between the different cut-offs in both assays can be achieved.

Previous studies on the relationship of ER content and prognostic factors have yielded heterogeneous data. While some investigators demonstrated a relationship,[25–27] others did not.[28–30]

In our series, there was a statistically significant correlation between the histologic tumor grade and the steroid receptor content with all methods of determination, except for PgR-DCC. Nuclear polymorphism as well as the rate of mitosis showed similar results. As for histologic grading, there was no correlation between the rate of mitosis and the progesterone receptor content determined by DCC.

Regarding the growth pattern, only PgR-ICA yielded a statistically significant correlation. In contrast to other groups[31,32] we found no correlation between estrogen receptor content and growth pattern. In agreement with other authors[33,34] we were able to demonstrate that nuclear features had a stronger impact on the prognosis of various subsets of breast cancer patients than the growth pattern.[35–38]

Our explanation for the controversial results of the progesterone correlation analysis with grading and several subfactors is only speculative. Controversial data may be due to technical problems caused by the higher susceptibility of the progesterone receptor to storage conditions, the preparation of cytosol and alterations of the epitope during the fixation procedure.

These problems could produce different results for the different assays. In any case, there was a trend towards a negative correlation, i.e., a higher percentage of progesterone receptor-positive tumors in the group with higher tumor differentiation.

In line with this, we found a lower percentage of PgR values > 10 fmol/mg cytosol than PgR-ICA values > 1 in the prognostically favorable groups (grading 1, low-grade nuclear polymorphism, mitosis rate 0–1/high-power field).

Controversial data have been reported with regard to the correlation of tumor size and receptor content. Berger[39] detected no correlation between receptor content and tumor size with ICA, while Neumann[40] found lower PgR-ICA values with increasing tumor size. Thorpe[41] saw a correlation of tumor size and ER content on DCC. Reiner[32] found a statistically significant negative correlation between tumor size and estrogen receptor positivity only with ER-ICA.

The aforementioned findings reflect our results, yielding a correlation exclusively for the biochemically determined estrogen receptor content. An indirect relationship via the correlation of tumor size and grading, as determined in our collective (data not shown), is conceivable. This, however, does not explain the isolated occurrence of this phenomenon encountered with ER-DCC.

Our data confirm the independence of the receptor content from the lymph node status, as already stated in various other reports[7,8,27,28,32] and suggest an independent prognostic validity of the hormone receptors for breast cancer.

The progesterone receptor is expressed irrespective of the menopausal status,[7,11] which is confirmed by our results. The statistically significant relationship between menopausal status and ER-ICA, as seen in our series, is a matter of controversy in several reports.[11,27,37,42] ER-DCC revealed the same tendency, without reaching statistical significance.

Lobular carcinoma revealed a high incidence of ER positivity with both methods. While this is in agreement with other findings with regard to ER-ICA[11,32] the incidence of ER positivity as determined with DCC for lobular carcinoma remains controversial.[25,28,29,31]

Contrary to Pertschuk, who reported preliminary data about a correlation between immunohistochemically determined PgR values and the histopathologic tumor type,[32] lobular carcinoma had an increased incidence of positivity in our series paralleled by a strikingly low incidence of receptor positivity with PgR-DCC.

Tubular carcinoma was commonly found to be receptor positive in our series in accordance with reports in the literature.[43,44] DCC yielded slightly lower PgR values, which seems to reflect an occasionally higher content of connective tissue in these tumors. The well-known tendency of medullary carcinoma towards receptor negativ-

ity was confirmed in our two patients.[25,28,29,44] However, the small collective excludes meaningful conclusions.

Mucinous carcinoma predominantly showed positivity, especially of ER, irrespective of the employed method of determination. Overall, ductal invasive carcinoma had the lowest incidence of positivity of all histologic groups with a relevant number of cases.

In theory, tumors with a high connective tissue content and thus low cellularity could produce discordant results (DCC-negative/ICA-positive).[16] We have therefore subject ductal invasive tumor samples with this tissue composition to separate investigation.

In our study this discordance was demonstrated for the progesterone receptor, without reaching statistical significance.

Our data confirm a high incidence of receptor-positive tumors in prognostically favorable subgroups, e.g., tubular carcinoma. This implication could not be substantiated with PgR-DCC, which proved to be the only method where no correlation with the histologic grading was observed. In patients with mucinous carcinoma, which is a prognostically favorable subgroup of breast cancer, we found a high rate of ER positivity with both methods. An evaluation of the PgR status was not possible due to the small number of cases.

To evaluate the respective role of both subfactors used in the IRS estimation, we performed a correlation analysis of both subfactors with the prognostic parameters as well as with histologic tumor types. While grading and nuclear features showed a highly significant correlation with both subfactors in all assays, tubular differentiation only revealed a significant negative correlation with PgR-ICA. This is in agreement with the fact that only in this assay there was a significant correlation of PP with a certain histologic tumor type, i.e., a significantly higher incidence of $< 10\%$ positive cells for ductal invasive NOS carcinoma versus all other histologic types.

The positive correlation between menopausal status and estrogen receptor expression also affects the subfactor correlation, where both subfactors demonstrate a significant relationship.

Interestingly, subfactor analysis revealed that the weak and statistically not significant negative correlation between tumor size and ER-ICA was based only on a statistically significant correlation with PP.

A summary of the subfactor correlation analysis might suggest the conclusion that PP has a stronger impact on IRS determination than SI. This is in agreement with prognostic analyses,[12,27,39] where PP represented a meaningful parameter with regard to the patient's outcome, whereas SI appeared to have no importance.

In conclusion, we were able to demonstrate that immunohistochemistry detected a closer correlation between prognostic factors and receptor data than biochemical analysis. This is in agreement with a rising number of reports confirming the superiority of ICA over DCC receptor determination as a predictive indicator of the outcome in breast cancer patients.

References

1. Knight WA, Livingstone RB, Gregory BJ, McGuire WL. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. Cancer Res 1977; 37:4669–4671.
2. Osborne CK, Yochmowitz MG, Knight WA, McGuire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 1980; 46:2884–2888.
3. Kinne DW, Butler JA, Kimmel M, Flehinger BJ, Menendez-Botet C, Schwartz M. Estrogen receptor protein of breast cancer in patients with positive nodes: high recurrence rates in the postmenopausal estrogen receptor-negative group. Arch Surg 1987; 122:1303–1306.
4. Shapiro CM, Schiffeling D, Bitran JD, et al. Prognostic value of the estrogen receptor value in pathologic stage I and II adenocarcinoma of the breast. J Surg Oncol 1982; 19:119–121.
5. Samaan NA, Buzlar AV, Aldinger KA, et al. Estrogen receptor: a prognostic factor in breast cancer. Cancer 1981; 47:554–560.
6. Butler JA, Bretsky S, Menendez-Botet C et al. Estrogen receptor protein of breast cancer as a predictor of recurrence. Cancer 1985; 55:1178–1181.
7. Clark GM, Osborne CK, McGuire WL. Correlations between progesterone receptor and patient characteristics in human breast cancer. J Clin Oncol 1984; 102:1102–1109.
8. Mc Guire WL, Clark GM. The prognostic role of progesterone receptors in human breast cancer. *In* International Journal of Cancer Research and Treatment Oncology: Management of Hormone-Responsive Tumors. Highlights of Symposium held in Monte Carlo, September 21, Basel: Karger 1983.
9. Greene GL, Fitch FW, Jensen EV. Monoclonal antibodies to estrophilin: probes for the study of estrogen receptors. Proc Natl Acad Sci USA 1980; 77:157–161.
10. Perrot-Applanat M, Logeat F, Croyer-Picard MT, Milgrom E. Immunocytochemical study of mammalian progesterone receptor using monoclonal antibodies. Endocrinology 1985; 116/4:1473–1483.
11. Pertschuk LP, Kim DS, Kamran Nayer MA, Feldman JG, Eisenberg KB, et al. Immunocytochemical estrogen and progestin receptor assays in breast cancer with monoclonal antibodies. Cancer 1990; 66:1663–1670.
12. Reiner A, Neumeister B, Spona J, Reiner G, Schemper M, Jakesz R. Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer. Cancer Res 1990; 50:7057–7061.
13. EORTC Breast Cancer Cooperative Group. Revision of the standards for the assessments of hormone receptors in human breast cancer. Eur J Cancer Clin Oncol 1980; 16:1513–1515.
14. Remmele W, Stegner HE. Vorschlag zur einheitlichen Definition eines immunreaktiven Score (IRS) für den immunhistochemischen Östrogenrezeptornachweis (ER-ICA) im Mammakarzinomgewebe. Pathologie 1987; 8:138–140.
15. WHO. The World Health Organization histological typing of breast tumors, 2nd ed. Am J Clin Pathol 1982; 78:806–816.
16. Beck Th, Pollow K, Grill HJ, Weikel W, Kreienberg R. Hormonrezeptornachweis der Mammakarzinome: additive Information immunhistochemischer und histologischer Untersuchungen zum biochemischen Rezeptorassay. Tumor, Diagnostik & Therapie 1989; 10:104–108.

17. International Union Against Cancer. TNM Classification of Malignant Tumors, 3rd ed. Geneva: UICC 1979; 4754.
18. Bloom HJG, Richardson WW. Histological grading and prognosis in breast carcinoma. Br J Cancer 1957; 11:359–377.
19. Kendall MG. Rank Correlation Methods, 3rd ed. London: Griffin, 1982.
20. Brown MB, Benedetti JK. Sampling behavior of tests for correlation in two-way contingency tables. J Am Stat Assoc 1977; 72:309–315.
21. Pertschuk LP. Immunocytochemistry for steroid receptors. Boca Raton: CRC Press (in press).
22. Kohail HM, Elias G, El-Nowiem SA, Bashirelahi N, Didolkar MS, Reed WP. A multifactorial analysis of steroid hormone receptors in stages I and II breast cancer. Ann Surg 1985; 201:611–617.
23. Saez S, Cheix F, Asselain B. Prognostic value of estrogen and progesterone receptors in primary breast cancer. Breast Cancer Res Treat 1983; 3:345–354.
24. Pertschuk LP, Feldman JG, Eisenberg KB, Carter AC, Thelmo WL, et al. Immunocytochemical detection of progesterone receptor in breast cancer with monoclonal antibody. Cancer 1988; 62:342–349.
25. Parl FF, Wagner RK. The histopathological evaluation of human breast cancers in correlation with estrogen receptor values. Cancer 1980; 46:362–367.
26. Fisher ER, Redmond CK, Liu H, Rockette H, Fisher B, and collaborating NSABP investigators. Correlation of estrogen receptor and pathologic characteristics of invasive breast cancer. Cancer 1980; 45:349–353.
27. Walker KJ, Bouzubar N, Robertson J, Ellis IO, Elston CW, Blamey RW, Wilson DW, Griffiths K, Nicholson RI. Immunocytochemical localization of estrogen receptor in human breast tissue. Cancer Res 1988; 48:6517–6522.
28. Rosen PP, Menendez-Botet CJ, Nisselbaum JS, et al. Pathological review of breast lesions analyzed for estrogen receptor protein. Cancer Res 1975; 35:3187–3194.
29. Lesser ML, Rosen PP, Senie RT, Duthie K, Mendendez-Botet C, Schwartz MK. Estrogen and progesterone receptors in breast carcinoma: Correlation with epidemiology and pathology. Cancer 1981; 48:299–309.
30. Thoresen S, Tangen M, Stoak F, Hartveit F. Estrogen receptor values and histological grade in human breast cancer. Histopathology 1981; 5:257–262.
31. Poulsen HS, Ozello L, Andersen J. Estrogen receptors in human breast cancer: problems of correlation with histopathological features. Virchows Arch (Pathol Anat) 1982; 397:103–108.
32. Reiner A, Reiner G, Spona J, Schemper M, Holzner JH. Histopathologic characterization of human breast cancer in correlation with estrogen receptor status. Cancer 1988; 61:1149–1154.
33. LeDoussal V, Tubiana-Hulin M, Friedman S, et al. Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson (SBR): an improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. Cancer 1989; 64:1914–1921.
34. Rank F, Dombernowsky P, Bang Jesperson NC, et al. Histologic malignancy grading of invasive ductal breast carcinoma: a regression analysis of prognostic factors in low-risk carcinomas from a multicenter trial. Cancer 1987; 60:1299–1305.
35. Stierer M, Rosen HR, Weber R. Histologic grading and subfactor analysis as prognostic parameters in patients with breast cancer. J Natl Cancer Inst 1990; 82:1510.
36. Rosen HR, Stierer M, Weber R. Nuclear polymorphism: a strong prognostic parameter to evaluate local recurrence of female breast cancer. Breast Cancer Res Treat 1990; 17:9–13.
37. Stierer M, Rosen H, Weber R. Nuclear pleomorphism, a strong prognostic factor in axillary node-negative small invasive breast cancer. Breast Cancer Res Treat 1991; 20:109–116.
38. Stierer M, Rosen HR, Weber R, Marczell A, Kornek GV, Czerwenka E. Factors influencing the outcome in breast cancer stage T1a and T1b: a monocenter long-term analysis. Surg Gynecol Obstet 1992; 175:151–160.
39. Berger U, McClelland RA, Wilson P, Greene GL, Haussler MR, et al. Immunocytochemical determination of estrogen receptor, progesterone receptor and 1,25-dihydroxyvitamine D3 receptor in breast cancer and relationship to prognosis. Cancer Res 1991; 51:239–244.
40. Neumann K, Rüschoff J, Horstmann J, Kolbfleisch H, Zwiorek L. Korrelation zwischen immunhistochemisch und biochemisch bestimmtem Progesteronrezeptorgehalt sowie Tumorgrading, Tumorausdehnung, Proliferationsrate und Chromatingehalt beim Mammakarzinom. Tumor Diagnostik Therapie 1989; 10:109–114.
41. Thorpe SM, Rose C, Rasmussen BB, Mouridsen HT, Bayer T, Keiding N. Prognostic value of steroid hormone receptors: Multivariate analyse of systemically untreated patients with node negative primary breast cancer. Cancer Res 1987; 47:6126–6133.
42. Nicholson RI, Campbell FC, Blamey RW, Elston CW, George WD, Griffiths K. Steroid receptors in early breast cancer: Value in prognosis. J Steroid Biochem 1981; 15:193–199.
43. Antoniades K, Spector H. Correlation of estrogen receptor levels with histology and cytomorphology in human mammary cancer. Am J Clin Pathol 1979; 71:497–503.
44. Mohammed RH, Lakatua DJ, Haus E, Yasmineh WJ, Estrogen and progesterone receptors in human breast cancer: correlation with histologic subtype and degree of differentiation. Cancer 1986; 58:1076–1081.